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Prolonged Delivery of BMP-2 Using Heparin-Derivatized Collagen Scaffolds

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Purpose: We evaluated a method to covalently link heparin oligosaccharides to collagen and enhance the binding of bone morphogenic protein-2 (BMP-2) to this derivatized surface. Since BMP-2 activity is potentiated in the presence of heparin, we hypothesize that BMP-2 loaded heparin-derivatized scaffolds will prolong BMP-2 release and amplify BMP-2 activity, thereby improving its ability to repair cartilage.
Methods and Materials: Heparin oligosaccharides (ff13 kDa) containing aldehyde groups were prepared by treating heparin with nitrous acid. These oligosaccharides were coupled to two collagen scaffolds, Helistat® and MAIX™, in the presence of sodium cyanoborohydride to yield heparin contents of 18% and 6% w/w, respectively.
Results: Table I shows that the in vitro release of BMP-2 was signifcantly slower for the heparin-derivatized scaffolds when compared to untreated matrices. Scaffold Untreated Heparinized Helistat 22% 4% MAIX 77% 16% Table I. Percent BMP-2 released from untreated versus heparin-derivatized collagen scaffolds over 14 days at 370C. BMP-2 eluted from a heparin-derivatized scaffold showed a 2-3 fold increase in alkaline phosphatase activity compared to growth factor eluted from controls. Implantation in a rat ectopic bone formation model showed that after 28 days, the degree of bone formation for heparin-derivatized BMP-2 scaffolds was similar to untreated scaffolds, with dense areas of bone localized at the implant margins.
Conclusions: Heparin-derivatized collagen scaffolds significantly slowed the release of BMP-2 while maintaining and potentially increasing growth factor activity. In vivo studies showed BMP-2 activity was maintained on heparinized scaffolds. Retention of BMP-2 and potential amplification of its bioactivity could be beneficial in a cartilage repair situation.

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Nanofibre scaffolds from pHEMA and PVA/Chitosan for cartilage tissue engineering

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Purpose: Nanofibers have a big potential in tissue engineering applications. Their topography is convenient for cell adhesion and proliferation. Poly(hydroxyethyl methacrylate), polyvinyl alcohol (PVA) and chitosan are biocompatible polymers widely used for medical practice. The aim of this work was to study chondrocyte proliferation and differentiation on these nanofibrous scaffolds.
Methods and Materials: Two kinds of scaffolds were prepared by electrospinning: scaffolds of copolymer of hydroxyethyl methacrylate and methacrylate sodium salt (pHEMA1/pHEMA2 samples using different crosslinking method), composite scaffolds of polyvinylalcohol (PVA) and chitosan, such as PVA/Chitosan1 and PVA/chitosan2 using different crosslinking method, and PVA/Chitosan3). Samples were seeded by rabbit chondrocytes and cultured for 7 days. Chondrocytes cultured on tissue culture plastic (TCP) were used as a control. Proliferation and viability of chondrocytes were tested using MTT test (on day 1, 3, and 7) and confocal microscopy; the presence of type II collagen was proved by immunofluorescence.
Results: MTT test showed good cell adhesion on all scaffolds. On day 3, the highest proliferation was observed on pHEMA1 and TCP, the lowest on PVA/Chitosan3. After 7 days, the lowest absorbance was observed on PVA/Chitosan3, all other scaffolds including TCP showed the same high MTT absorbance. Good correlation between propidium iodide staining and MTT test was observed. Immunofluorescence proved presence of type II collagen on all scaffolds.
Conclusions: Samples made from pHEMA and PVA/Chitosan with higher amount of chitosan (PVA/Chitosan1, PVA/Chitosan2) showed excellent chondrocyte proliferation. These scaffolds can be potentially used for cartilage repair.

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Redifferentiation and Hyaline-Specific Extracellular Matrix Protein Synthesis in Human Chondrocytes Cultured in a PCL Scaffold

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Purpose: The redifferentiation, proliferation and hyaline-specific extracellular matrix (ECM) protein synthesis of chondrocytes cultured in a polycaprolactone (PCL) scaffold were analysed.
Methods and Materials: Gene expression of type II collagen and aggrecan was assessed (PCR) in cells from PCL, monolayer and pellet cultures. Ki-67 immunodetection was used to assess the proliferative activity of cells cultured. Chondrocytic differentiation was evaluated using S-100 immunodetection, and the synthesis, and deposition into scaffold pores, of type II collagen and glycosaminoglycan (GAG) were analysed by immunohistochemistry and Alcian blue staining, respectively. All parameters were assessed at 7, 14 and 28 days of cultures maintained in either FBS-containing medium (FCM) or ITS-containing medium (ICM).
Results: Expression of type II collagen gene was lower in FCM than in ICM for all culture systems ($p<0.05$) at all times of measure. PCL scaffolds cultured in ICM were able to induce collagen gene expression more efficiently than pellet and monolayer cultures. Aggrecan gene expression did not vary significantly among FCM cultures. In ICM, the monolayer cultures had significantly higher levels of aggrecan gene expression than did either the PCL or pellet cultures. Chondrocytes in PCL scaffolds or pellets with FCM did not proliferate to a great extent but maintain their differentiated phenotype for 28 days. Levels of hyaline-specific ECM protein synthesis, and protein deposition into the scaffold pores, were similar among PCL and pellet cultures grown in FCM and in ICM.
Conclusions: Chondrocytes seeded in PCL scaffolds and cultured in medium supplemented with ITS and ascorbate, maintained their phenotype and were able to synthesise specific proteins.

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Release kinetics and bioactivity of rhBMP-2 on collagen scaffolds: application to cartilage repair

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Purpose: BMP-2 has been shown to direct marrow-derived mesenchymal stem cells toward chondrogenic differentiation. We hypothesize that delivery of recombinant BMP-2 to a microfractured cartilage lesion will improve tissue repair, and that different delivery vehicles could modulate the duration of exposure of joint tissues to BMP-2 stimulation.
Methods and Materials: We used in vitro and in vivo models to study BMP-2 elution kinetics and bioactivity after lyophilization onto three collagen scaffolds with distinct physical characteristics and formulations.
Results: In a 14-day in vitro study, BMP-2 elution depended on the scaffold employed, with more dense scaffolds releasing BMP-2 more slowly, and to a lesser overall extent. Bioactivity was assessed by cell-based assay, with an average of 81% of the released protein remaining active, and no differences among scaffolds. Bioactivity was also evaluated in vivo using BMP-2 loaded collagen scaffolds in a rat subcutaneous implant model. Although all scaffolds supported BMP-2-induced bone formation (indicating active BMP-2), the extent and spatial distribution of bone deposition varied among the scaffolds. Cell infiltration increased with scaffold porosity, with a higher total mineral deposition and a more homogeneous distribution over the 28-day implantation period in more porous scaffolds.
Conclusions: Lyophilizing BMP-2 onto collagen scaffolds resulted in different release kinetics related to the scaffold physical properties, with overall maintenance of BMP-2 activity. Our preparation resulted in an easy-to-handle, unitary device suitable for implantation. Ongoing studies are investigating chemical modulation of scaffolds to alter the BMP-2 elution rate, and testing these scaffolds in large animal models of cartilage repair.